REMARKS

1. Applicants hereby submit the following:

[ ] a paper copy of a "Sequence Listing", complying with §1.821(c), to be incorporated into the specification as directed above;

[XX] an amendment to the paper copy of the "Sequence Listing" submitted on July 27, 2000, the amendment being in the form of substitute sheets;

[XX] the Sequence Listing in computer readable form, complying with §1.821(e) and §1.824, including, if an amendment to the paper copy is submitted, all previously submitted data with the amendment incorporated therein;

[ ] pursuant to §1.821(e), reference is made to the computer readable form filed on , in USSN , which presents the identical Sequence information, the use of which is now requested, in lieu of submitting a new computer readable form; and/or

[ ] a substitute computer readable form to replace one found to be damaged or unreadable.

[XX] 2. The description has been amended to comply with §1.821(d).

3. The undersigned attorney or agent hereby states as follows:

(a) this submission is not believed to include new matter [§1.821(g)];

(b) the contents of the paper copy (as amended, if applicable) and the computer readable form of the Sequence Listing, are believed to be the same [§1.821(f) and §1.825(b)];

(c) if the paper copy has been amended, the amendment is believed to be supported by the specification and is not believed to include new matter [§1.825(a)]; and

(d) if the computer readable form submitted herewith is a substitute for a form found upon receipt by the PTO to be damaged or unreadable, that the substitute data is believed to be identical to that originally filed [§1.825(d)].

5. Under U.S. rules, each sequence must be classified in <213> as an "Artificial Sequence", a sequence of "Unknown" origin, or a sequence originating in a particular organism, identified by its scientific name.

Neither the rules nor the MPEP clarify the nature of the relationship which must exist between a listed sequence and an organism for that organism to be identified as the origin of the sequence under <213>.

Hence, counsel may choose to identify a listed sequence as associated with a particular organism even though that sequence does not occur in nature by itself in that organism (it may be, e.g., an epitopic fragment of a naturally occurring protein, or a cDNA of a naturally occurring mRNA, or even a substitution mutant of a naturally occurring sequence). Hence, the identification of an organism in <213> should not be construed as an admission that the sequence *per se* occurs in nature in said organism.

Similarly, designation of a sequence as "artificial" should not be construed as a representation that the sequence has no association with any organism. For example, a primer or probe may be designated as "artificial" even though it is necessarily complementary to some target sequence, which may occur in nature. Or an "artificial" sequence may be a substitution mutant of a natural sequence, or a chimera of two

or more natural sequences, or a cDNA (i.e., intron-free sequence) corresponding to an intron-containing gene, or otherwise a fragment of a natural sequence.

The Examiner should be able to judge the relationship of the enumerated sequences to natural sequences by giving full consideration to the specification, the art cited therein, any further art cited in an IDS, and the results of his or her sequence search against a database containing known natural sequences.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "Version with markings to show changes made".

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the specification:

The paragraph beginning at line 14 of page 21 has been amended as follows:

The inserts of all 8 clones were confirmed to be PBGD by complete double strand sequence analysis (see ~~sequences 1 - 8~~SEQ ID NOs:1-8). Each has some change(s) from the published sequences. Some changes are unique and some are shared with other clones (see Table 2 and Table 3). For differences found only in one clone, it is difficult to distinguish between PCR or cloning artifacts and actual allelic variations without additional sampling. However, when the same base difference is found in more than one sequence it is unlikely to be from cloning errors. From the alignment of all 11 PBGD sequences a set of common bases emerged, the consensus or wild type allele sequence. Five of the eight clones (1.1, 1.3, 2.1, 3.3, and 5.3.) have the wild type amino acid sequence. Within this set with wild type amino acid sequence, there is only one difference at the nucleic acid level. At position 555, 4 of the 5 sequences have a dGTP while 1 along with the published erythropoietic and genomic PBGD have a dTTP. These appear to be two common alleles, which result in no amino acid difference. There are 2 base changes between clone number 1.1 and the published

erythropoietic PBGD. An adenine to guanine change at base 513 (Leu 171) is a silent mutation, which is also present in 9 out of the 11 sequences, compared. The second difference is a cytosine to adenine substitution at base 995 (Thr 332.) This is not a silent change, with a threonine to asparagine non-conservative mutation. It appears however that the difference is an error in the published erythropoietic PBGD sequence since all 10 other sequences have an adenine at this position. In addition to these natural variations, there are three additional silent mutations introduced during the cloning at positions 1017, 1018 and 1020 to create a Mun-I site for future manipulations. The PBGD gene was ligated into pBluescript SK plasmid generating the pSK-PBGD 3988 bp plasmid, which was sequenced (see Fig. 1, Fig. 9a-9x and ~~sequence 9~~SEQ ID NO:9).

Table 1 of page 23 has been amended as follows:

Table 1 Oligonucleotide primers:

Ico375-pbgds (32 mer) coding region 5′ end w/ EcoRI site sense
5′ CGT G**GA ATT C**AT GAG AGT GAT TCG CGT GGG TA 3′ (SEQ ID NO:13)

Ico376-pbgda (47 mer) coding region 3′end w/ HindIII site antisense
5′ GGA G**AA GCT T**AT TAA TGG GCA TCG TTC AAT TGC CGT GCA ACA TCC AG 3′
(SEQ ID NO:14)

Ico378-csnonc (20 mer) constitutive form non-coding sense
5′ TCC AAG CGG AGC CAT GTC TG 3′ (SEQ ID NO:15)

Ico379-esnonc (20 mer) erythropoietic form non-coding sense
5′ TCG CCT CCC TCT AGT CTC TG 3′ (SEQ ID NO:16)

Ico380-sinter (21 mer) internal coding sense

5′ CAG CAG GAG TTC AGT GCC ATC 3′ (SEQ ID NO:17)

Ico381-ainter (21 mer) internal coding antisense
5′ GAT GGC ACT GAA CTC CTG CTG 3′ (SEQ ID NO:18)

Ico382-anonc (20 mer) non-coding sense
5′ CAG CAA CCC AGG CAT CTG TG 3′ (SEQ ID NO:19)

Ico383-pSKT7 (22 mer) pBluescript T7 promoter
5′ GTA ATA CGA CTC ACT ATA GGG C 3′ (SEQ ID NO:20)

Ico384- pSKpjrev(22 mer) pBluescript reverse1
5′ CTA AAG GGA ACA AAA GCT GGA G 3′ (SEQ ID NO:21)

Ico385- pSKrev (21 mer) pBluescript reverse2
5′ CAG CTA TGA CCA TGA TTA CGC 3′ (SEQ ID NO:22)

The paragraph beginning at line 12 of page 29 has been amended as follows:

AATTCTAACA TAAGTTAAGG AGGAAAAAAA A ATG AGA GTT ATT CGT GTC GGT AC (SEQ ID NO:23)

Met Arg Val Ile Arg Val Gly (SEQ ID NO:24)

Plasmid pExp1 (Fig. 5) was made in a two step process. Oligonucleotides ICO386 (5′ AAT TCT AAC ATA AGT TAA GGA GGA AAA AAA AAT GAG AGT TAT TCG TGT CGG TAC 3′ (SEQ ID NO:25)) and ICO387 (5′ CGA CAC GAA TAA CTC TCA TTT TTT TTT CCT CCT TAA CTT ATG TTA G 3′ (SEQ ID NO:26)) were designed to provide upon annealing a 5′ *Eco*R I adhesive end and a 3′ *Kpn* I sticky end. Oligonucleotides ICO386 and ICO387 were annealed and ligated with the *Kpn* I-*Hin*d III PBGD fragment from pPBGD1.1 into *Eco*R I -*Hin*d III linearized pBluescript II SK- (Stratagene Cat #

212206) to yield plasmid pPBGD1.1Tra (Fig. 6). In the second step the *Eco*R I -*Hin*d III fragment from pPBGD1.1Tra was ligated into pKK223-3 cut with the same enzymes resulting in plasmid pExp1 (Fig. 5).

The paragraph beginning at line 12 of page 30 has been amended as follows:

The following strategy was used. Plasmid pExp1 was cut with *Sal* I and *Bam*H I and the 5348 base-pair fragment containing part of the tetracycline coding sequence and the bulk of the plasmid was isolated. Into this was ligated the *Sal* I-*Hin*d III fragment from pBR322 (New England BioLabs Cat # 303-3S, Lot # 50) containing rest of the coding sequence and an adapter formed by annealing oligonucleotides ICO424 (5' GATCACTCAT GTTTGACAGC TTATCATCGA TT 3' (SEQ ID NO:27)) and ICO425 (5. AGCTAATCGA TGATAAGCTG TCAAACATGA GT 3' (SEQ ID NO:28)). The adapter contains part of the tetracycline promoter and provides *Hind* III and *Bam*H I overhangs for ligation but destroys the *Hin*d III and *Bam*H I restriction sites.

The paragraph beginning at line 24 of page 31 has been amended as follows:

AATTCTAACA TAAGTTAAGG AGGAAAAAAA A ATG AGA GTT ATT CGT GTC GGT AC

(SEQ ID NO:23)

Met Arg Val Ile Arg Val Gly

(SEQ ID NO:24)

Oligonucleotides ICO386 (5′ AAT TCT AAC ATA AGT TAA GGA GGA AAA AAA AAT GAG AGT TAT TCG TGT CGG TAC 3′ (SEQ ID NO:25)) and ICO387 (5′ CGA CAC GAA TAA CTC TCA TTT TTT TTT CCT CCT TAA CTT ATG TTA G 3′ (SEQ ID NO:26)) were designed to provide upon annealing a 5′ *Eco*R I adhesive end and a 3′ *Kpn* I sticky end. Oligonucleotides ICO386 and ICO387 were annealed and ligated with the *Kpn* I-*Hin*d III PBGD fragment from pPBGD1.1 into *Eco*R I -*Hin*d III linearized pBluescript II SK- (Stratagene Cat # 212206) to yield plasmid pPBGD1.1Tra. In the second step the *Eco*R I -*Hin*d III fragment from pPBGD1.1Tra was ligated into pKK223-3 cut with the same enzymes resulting in plasmid pExp1.

The two paragraphs beginning at line 3 of page 32 and ending at line 20 of page 32 have been amended as follows:

Plasmid pExp1 was cut with *Sal* I and *Bam*H I and the 5348 base-pair fragment containing part of the tetracycline coding sequence and the bulk of the plasmid was isolated. Into this was ligated the *Sal* I-*Hin*d III fragment from pBR322 (New England BioLabs Cat # 303-3S, Lot # 50) containing rest of the coding sequence and an adapter formed by annealing

oligonucleotides ICO424 (5′ GATCACTCAT GTTTGACAGC TTATCATCGA TT 3′ (SEQ ID NO:27)) and ICO425 (5. AGCTAATCGA TGATAAGCTG TCAAACATGA GT 3′ (SEQ ID NO:28)). The adapter contains part of the tetracycline promoter and provides *Hind* III and *Bam*H I overhangs for ligation but destroys the *Hin*d III and *Bam*H I restriction sites. The resulting plasmid was called pExp1-M2.

Plasmid pExp1-M2 was digested with Pvu I and Afl III and the larger of the two fragments corresponding to a size of 4745 base-pairs was isolated. This was ligated to the 1257 base-pairs long Pvu I-AflIII fragment derived from pUC19 containing the origin of replication and part of the ampicillin resistance gene to obtain plasmid pExp1-M2-Puc. This was passaged through JM110 and cut with BsaA1 and BsaB1 to excise the rom gene contained between the two sites and blunt-ended together to yield the final expression plasmid pExp1-M2-Puc-BB. The pExp1-M2-Puc-BB plasmid has been fully sequenced (see ~~sequence~~ SEQ ID NO:11).

The paragraph beginning at line 8 of page 42 has been amended as follows:

Met-Ser-Gly-Asn-Gly-Asn-Ala-Ala-Ala-Thr-Ala-Glu-Glu-Asn-Ser-Pro-Lys-**Met-Arg-Val**….(SEQ ID NO:30)

ATG-TCT-GGT-AAC-GGC-ATT-GCG-GCT-GCA-ACG-GCG-GAA-GAA-AAC-AGC-CCA-AAG-ATG-AGA-GTG..(SEQ ID NO:29)

The paragraph beginning at line 24 of page 47 has been amended as follows:

Normal Chromosomal Sequence:

5′-AG CGC ATG GGC TGG CAC AAC CGG GT-3′ (SEQ ID NO:31)

Gln Arg Met Gly **Trp** His Asn Arg Val (SEQ ID NO:32)

AIP Chromosomal Sequence:

5′- AG CGC ATG GGC TAG CAC AAC CGG GT-3′ (SEQ ID NO:33)

Stop

The paragraph beginning at line 1 of page 48 has been amended as follows:

The sequence of the chimeric oligonucleotide (Heme593W/X) is: